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Synopsis submitted on

"Studies on Biologically Active Compounds Produced by Certain Members of Non-Heterocystous Cyanobacteria"

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Introduction

Cyanobacteria generally remain potential source for investigations as prospective and excellent sources of biologically active constituents produced during primary and especially secondary metabolism. Primary metabolites are allow molecular weight compounds that are necessary for growth, these are produced by microorganisms during active growth, which include amino acids. Nucleotides, coenzymes, organic acids and vitamins where as secondary metabolites are not necessary for microbial growth.

Among microorganisms, Cyanobacteria are rapidly proving to be an extremely important source of biologically active secondary metabolites with potential benefits against human diseases. They have been shown to produce a variety of antibacterial, antifungal, antilarval,. antiprotozoan, antialgal, antihelmintic and cytotoxic secondary metabolite (Meeting and Pyne, 1986; Glombitza and Koch, 1989). Some of which have potential for use as therapeutic or agrochemical agents (Moore 1996)

Cyanobacteria toxins are most well studied, and largely associated, with regard to their effects on human and environment health. Indeed., toxicity of Cyanobacteria metabolites was first reported in the scientific literature by George Francis following death of livestock in south Australia. After consumption of Cyanobacteria contaminated drinking water from Lake Alexandria. Since then, a number of incidents of human and non-human

animal poisoning has been described and several good references on the human health effects of Cyanobacteria toxins have been previously published. New antibiotics with high activity and without side effects for human and for environment are therefore urgently needed. Some Cyanobacteria species could be a prolific resource for substances with antibacterial activity.

Review of Literature:

Schlegel *et al* (1999) reported that microorganisms may produce more than one bioactive substances targeted against different biochemical processes. Cyanobacteria are evolved under anoxic conditions and are well adapted to environmental stress including exposure to ultraviolet radiations, high solar radiation and temperatures, scarce and abundant nutrients. Cyanobacteria is widely studied, not only for nutritional reasons, but also for its extracts could precent or inhibit cancer in humans and animals, and recent works have indicated that this species has immune-promoting effects. (Qureshi and Ali 1996; Hayakawa *et al*. 1997; Kim *et al*. 1998; Miranda *et al*. 1998; Mishima *et al*. 1998; Hirahashi *et al*. 2002; Subhasini *et al* 2004). *Spirulinaplatensis* was reported to present antimicrobial activity (Demule *et al. 1*996; Ozdemir *et al*. 2004) as well as to inhibit the replication of several viruses. Such as Herpes simplex and HIV-1 (Ayehunie *etal*. 1998; Hernandez-corona *et al*. 2002).

There are numerous reports concerning the inhibiting activities from cyanobacterial against human pathogens (Kreitlow et al, 1999; Abdel-Raouf 2007), Fungi (Moussa and Shahab 2001; Ibraheem and Mohammed 2002; Hussan 2007; Sunil and Puranik 2007), mites (Ibraheem and Abdel-Aziz 2002), algae (Issa, 1999 and Volk and Furkert, 2006) but there is data about effects

against human pathogenic bacteria and fungi. Cyanobacteria are prokaryotic photoautotrophs capable of carrying out photosynthetic and nitrogen fixation simultaneously

Certain cyanobacteria have drown much attention as prospective and rich sources of biologically active constituents and have been identified as one of the most promising croups of organism capable of producing bioactive compounds (Fish and Cold. 1994 and Schlegel *etal*, 1999)

Objectives of the study:

1 Identification and characterisation of Non-heterocystous cyanobacteria

2 To study the Cyanobacteria toxins for the development of biologically active compounds such as fungicides, insecticides, algaecides and herbicides

3 The present study is undertaken to evaluate biotechnological potentials of Cyanobacteria for obtaining pharmacologically active secondary metabolites.

4 Main Goal of present research work is to focus on screening of antimicrobial compounds of cyanobacteria found in warangal district of Telangana state which may prove to be an effective ecofriendly approach for Bacterial and Fungal diseases

5 From pharmaceutical point of view cyanobacteria may produce good source of new the bioactive compounds.

Research Plan :

The paddy field' s non heterocystous Cyanobacteria of Warangal district, Telangana state have not been studied for their antimicrobial activity so far. Hence in the present study our interest is to work on isolation of C-phycocyanin pigment which has particular applications in various fields and antimicrobial activity of some species of non heterocystous Cyanobacteria namely *Chroococcus minor, Lynghya ceylanica, Microcystis aerugnosa, Oscillatoria acuta and Phormidium corium* against pathogenic organisms like *Bacillus subtilis, Staphylococcus aureus, Streptococcus mutants, Escherichia coli, Micrococcus mutant, Klebsiella pneunoniae, Saccaromyces cerevisiae* and *Candida albicans*. The main aim of the study will be on bacteriostatic activity, pharmacological diagnosis of above mentioned cyanobacteria of warangal district of Telangana State.

Materials and Methods:

Soil samples will be collected from agro-climatic regions of paddy fields of warangal district in North Telangana region of TS. These samples can be cultured in BG 11 media at Incubation temp 28^{O}C and illumination 3000 lux with a continuous white fluorescent light.

Identification: For identification of forms at generic and species level the schemes and characters proposed by Anand (1989), Desikachary (1959), Pandey (1965), Santra (1993), Tiwari (1972) methods can be used in this work.

AntimicrobialBioassay:

Antimicrobial activities of the extraction solvent of *Chroococcusminor, Lyngbya ceylanica, Microcystis aeruginosa, Oscillatoria acuta and Phormidium corium* against pathogenic organisms like *Bacillus subtilis, Staphylococcus aureus,*

Streptococcus mutants, Escherichia coli, Micrococcus mutants, Klebsiella pneumoniae, Saccaromyces cerevisiae and *Candida albicans* can be determined by the paper disk diffusion method. In this method, the filter paper discs (6mm) were saturated with 50*u*l of the test solution, dried under laminar air flow and placed on the Saubouraud' s dextrose agar plate for fungal strains and the Muller Hinton Media (Hi-Media) for Bacterial Strains, which had been inoculated for 24 hours at 37ºC for bacteria and inoculated for 3 day sat 30ºC for fungi. Discs treated with 50*u*l, respective solvents (Ethanol, Acetone, Diethyl ether and Methanol) can be used as negative control and to study the antimicrobial activity of cyanobacteria with standard antibiotics (Erhthromycin, Tetracycline, and Amoxycillin) and fungicides (Itraconazole and Polyoxylin) discs were used (10*u*g) as positive controls. The Cyanobacteria extracts containing antifungal compounds is expected to produce distant, Clear, circular zones of inhibition around the discs and the diameters of clear zones can be determined in millimetres and can be used as an indication of antifungal activity. All tests can be made in triplicate under sterile conditions.

Medium:

BG 11 medium composition

For 1 litre media:

1. To approximately 900 ml of dH_2O and the components in the order specifies while stirring continuously
2. Bring total volume to 1 L with dH_2O
3. Cover and autoclave medium

4. Allow to cool then store at refrigerator temperature

Agar media:

To approximately 400 ml of dH_2O add the components in the order specified while stirring continuously

5. Bring total volume to 500 ml with dH_2O
6. In a separate container add15 gm of agar to 500ml of dH_2O
7. Cover and autoclave both solutions
8. In a water bath allow both solutions to cool to 45-50^{O}C.
9. Add sterile sodium thiosulphate to agar solution and maxwell
10. Combine both agar and liquid solutions, mix well.
11. Allow to cool then store at refrigerator temperature.

Method of sampling:

Visible masses of BGA samples can be collected in plastic vials, containing growth medium (BG 11 medium) and brought to laboratory for further microscopic observations and culturing. The samples can be fixed in 4% formalise and the rest was used for isolation and culturing. Collection of soil samples could be done from the soil surface in plastic bags, brought to the laboratory and air dried.

The present plan of work is divided into five different steps like

	Isolation and Identification of non-heterocystous cyanobacteria
3	Taxonomical Identification
4	Growth and preservation of cyanobacteria
5	Isolation of C-phycocyanin Pigment
6	Antimicrobial activity of Cyanobacteria extracts.

Isolation of phycocyanin pigment:

The cultures can be harvested after 30 days by centrifugation at 5000 rpm for 15 min. The algal pellet can be extracted with 15 ml of Acetone with shaking for 20 minutes. The culture supernatants and solvent extracts were dried under reduced pressure at $40^{O}C$ and were stored at $-10^{0}C$ for further studies. The most commonly used method for the extraction of phycobiliprotein C-phycocyanin can be isolated following the method of Boussiba and Richmond (1979) and estimation as described by Bennet and Bogorad (1973).

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